Exhibit 3

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包含CLORETAZINETM的治療組合物
COMBINATION THERAPY COMPRISING CLORETAZINETM

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(54) Title: COMBINATION THERAPY COMPRISING CLORETAZINE™

(57) Abstract: This invention provides a method for treating tumor in a subject comprising administering to the subject an effective amount of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog. This invention also provides a method for inhibiting tumor cell growth comprising contacting the tumor cell with effective amounts of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog. The present invention relates to the treatment of cancer, comprising administering to a subject in need thereof an effective amount of VNP40101M in combination with a nucleoside.

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COMBINATION THERAPY COMPRISING CLORETAZINE™

This application claims benefit of U.S. Serial No. 60/556,565, filed March 26, 2004. The content of this preceding application is hereby incorporated in its entirety by reference into this application.

Throughout this application, various publications are referenced. Disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

FIELD OF THE INVENTION

This invention relates to combination therapy comprising Cloretazine™. This invention further relates to synergistic effects of Cloretazine™ and nucleosides.

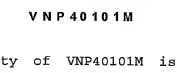
BACKGROUND OF THE INVENTION

Alkylating agents are among the most effective therapeutic agents currently available to treat different malignancies, and are widely used in the clinic (Katzung, In Basic & Clinical Pharmacology, 7th edition, 1998, Appleton & Lange, Stamford, 881). The high degree of cytotoxicity is attributed to the ability to induce DNA interstrand cross-linking thereby inhibiting replication (Rajski and Williams, Chem Reviews 1998, 98: 2723). Among the alkylating agents, the CNU (chloroethylnitrosourea) series have been widely used clinically to treat brain tumors, colon cancer and lymphomas (DeVita, et al. Cancer Res. 1965, 25: 1876; and Nissen, et al. Cancer 1979, 43: 31), however, their clinical usefulness is limited due to delayed and cumulative bone marrow depression and hepatic toxicity (Panasci, et al. Cancer Res. 1977, 37: 2615; and Gibson and Hickman, Biochem Pharmacol. 1982, 31: 2795).

A series of 1,2-bis(sulfonyl)hydrazine prodrugs (SHPs) with the ability to generate chloroethylating and carbamoylating species,

but lacking hydroxyethylating and vinylating species, generated by the CNUs had been developed recently (Sartorelli, et al. see US patent no. 6,040,338; US patent no. 5,637,619; US patent no. 5,256,820; US patent no. 5,214,068; US patent no. 5,101,072; US patent no. 4,849,563; and US patent no. 4,684,747). The antitumor activity has been suggested to result from chloroethylating and subsequent cross-linking of DNA (Kohn, In Recent Results in Cancer Research, Eds. Carter, et al., 1981, Springer, Berlin, vol. 76: 141; and Shealy, et al., J Med Chem. 1984, 27: 664). The carbamoylating species (i.e., the isocyanate) can react with thiol and amine functionalities on proteins and inhibit DNA polymerase (Baril, et al. Cancer Res. 1975, 35: 1), the repair of DNA strand breaks (Kann, et al. Cancer Res. 1974, 34: 398) and RNA synthesis and processing (Kann, et al. Cancer Res. 1974, 34: 1982). However, hydroxyethylation of DNA is a carcinogenic and/or mutagenic event (Swenson, et al. J Natl Cancer Inst. 1979, 63: 1469).

1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-(methylaminocarbonyl) hydrazine (VNP40101M, Cloretazine™), the current lead compound in the SHP series, has lower toxicity to hosts and better anti-tumor activities against the L1210 murine leukemia, L1210/BCNU, L1210/CTX, L1210/MEL (1,3-bis(2-chloroethyl)-1-nitrosourea, cyclophosphamide and melphalan resistant sublines), P388 leukemia, M109 lung carcinoma, B16 melanoma, C26 colon carcinoma and U251 glioma than chloroethylnitrosourea (CNU) derivatives and other SHP analogs (Shyam, et al. J Med Chem. 1999, 42: 941). In addition, VNP40101M is effective in crossing the blood brain barrier (BBB) and eradicating leukemia cells implanted intracranially (> 6.54 log cell kill), rivaling the efficacy of BCNU (Finch, et al. Cancer Biochem Biophys. 2001, 61: 3033).



VNP40101M

The anti-tumor activity of VNP40101M is probably due to the release of 90CE and methyl isocyanate. 90CE further fragments to

yield methyl 2-chloroethyldiazosulfone (1), see Figure 1 of US Patent no. 6,855,695, a relatively specific O^6-guanine chloroethylator, producing minimal alkylation of the N^7-position of guanine (Penketh, et al. J Med Chem. 1994, 37: 2912; and Penketh, et al. Biochem Pharmacol. 2000, 59: 283). Methyl isocyanate released from VNP40101M has the ability to inhibit various DNA repair enzymes including O^6-alkylguanine-DNA alkyltransferase leading to stabilization of the O^6-alkylguanine monoalkyl species in DNA, which leads to a larger percentage of interstrand cross-links (Baril, et al. Cancer Res. 1975, 35: 1).

Activity in Murine Tumor Models

VNP40101M has shown broad anti-tumor activity against leukemia and solid syngeneic and human xenograft tumors in murine models (Shyam et el., J Med Chem 28:525-7, 1985; Shyam et al., J Med Chem 29:1323-5, 1986; Shyam et al., J Med Chem 30:2157-61, 1987; Shyam et al., J Med Chem 36:3496-502, 1993; Shyam et al., J Med Chem 39:796-801, 1996). The data is summarized briefly below:

1. Against intraperitoneal (IP) implanted L1210 leukemia (10^6 tumor cells) that is resistant to 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), cyclophosphamide, or melphalan, a single dose of VNP40101M administered IP 24 hours after tumor inoculation (20-60 mg/kg, or 60-180 mg/m^2) was curative in 100% of mice (survival ≥ 60 days). The VNP40101M doses required to produce long-term survival in sensitive and resistant L1210-bearing mice were only modestly myelosuppressive when administered to non-tumor-bearing mice. Higher single doses (80-100 mg/kg) produced signs of a wasting condition and death in some animals, which occurred > 50 days after treatment. When administered IP daily for 6 days, VNP40101M at a dose of 6 mg/kg/d (total dose 36 mg/kg) increased life span of treated mice by 333% compared to control mice, and at doses of 12-18 mg/kg/d (total doses of 72-108 mg/kg) was curative in 100% of animals. Delayed toxic deaths were not observed with the d x 6 schedule, however, total doses higher than 108 mg/kg were not tested.

2. Against subcutaneous tumors of the human U251 glioma staged to ~300 mg in nude mice, VNP40101M administered as 10 mg/kg q2d x

11 or 20 mg/kg q4d x 6 IP caused complete regressions.

3. Against the murine M109 lung carcinoma implanted subcutaneously and staged to ~150 mg, VNP40101M administered as 10 mg/kg q2d x 10 or 20 mg/kg q4d x 5 IP delayed tumor growth, with the latter schedule delaying time to reach 1 gram by 14 days.
4. Administration of VNP40101M weekly by the IP route produced significant delays in tumor growth against the syngeneic B16F10 melanoma and against the human HTB177 lung (H460) and WiDr colon carcinoma cell lines. Doses from 10-60 mg/kg were active, but higher doses produced greater growth inhibition.
5. Substantial anti-tumor activity was observed by IP administration of VNP40101M against intra-cranially implanted L1210 leukemia, demonstrating good penetration through the blood-brain barrier.

Toxicology Studies

The relationship between VNP40101M dose and white blood cell count (WBC) was examined in normal CD_2F_1 mice. Modest leukopenia (50% of baseline) was observed with a single IP dose of 40 mg/kg (120 mg/m^2). VNP40101M doses of 60-80 mg/kg reduced WBC counts to approximately 25 and 15% of baseline, respectively, by day 4 with full recovery by day 21. As noted in section 1.2.A, high single doses of 80-100 mg/kg administered IP to tumor-bearing mice produced signs of a wasting condition and deaths occurring > 50 days after treatment.

Toxicology studies were performed in rats. A dose of 3 mg/kg (18 mg/m^2), when given intravenously (IV) on a d x 5 dosing schedule, produced no clinical signs or symptoms on day 15, but 2/10 rats had lung findings on day 29, including a small amount of thoracic cavity fluid and failure of the lung to collapse. Microscopic findings at the 3 mg/kg (d x 5) dose level were primarily limited to the lung and included alveolar edema, congestion, alveolar histiocytosis, and vascular thrombi. The higher dose of 10 mg/kg (60 mg/m^2) d x 5 produced few significant gross necropsy findings on day 15, but thoracic cavity fluid was found in approximately 50% of animals sacrificed on day 29 and 6/6 animals sacrificed on days 30/31. Histopathologic findings in the lung were similar to those observed at the 3 mg/kg dose level. For doses as high as 10

mg/kg d x 5, myelosuppression was not observed. Effects on serum chemistries were limited to decreases in total protein and albumin, which were observed on day 29 in the 10 mg/kg dose group.

In toxicology studies performed in dogs, single doses of 1, 3, 10, and 30 mg/kg were administered intravenously. The 1 and 3 mg/kg doses were well-tolerated and produced minimal clinical signs through at least 21 days of observation. The higher doses of 10 and 30 mg/kg (200 and 600 mg/m^2, respectively) produced marked clinical signs, as well as laboratory abnormalities including increased alkaline phosphatase, decreased albumin, increased bilirubin, increased creatine phosphokinase, and decreased white and red blood cell counts. Toxicity was also assessed for 0.3, 1 and 3 mg/kg doses administered intravenously daily x 5. The 3 mg/kg d x 5 dose produced marked clinical signs including reduced activity, loose stool, anorexia and slight dehydration, requiring sacrifice of the animals on day 8. There was also marked leukopenia by day 8, and slight elevation of the alkaline phosphatase. A dose of 1 mg/kg (20 mg/m^2) d x 5 produced minimal clinical signs and symptoms, and only a mild leukopenia on day 8 that recovered to baseline by day 15.

Phase I Studies of VNP40101M

VNP40101M has been studied in two phase I trials conducted in patients with advanced solid tumors or hematologic malignancies. In the first phase I trial, 26 patients with solid tumors were treated by IV infusion over 15-30 minutes at dose levels ranging from 3-305 mg/m^2 every 4-6 weeks. The maximum tolerated dose (MTD) was 305 mg/m^2. Among the seven patients treated at the MTD, six developed grade 3 thrombocytopenia. The platelet nadir occurred between days 25-33. Five of the patients treated at the MTD developed $\geq$ grade 2 granulocytopenia, but only one patient had a grade 3 event. Hematologic toxicities recovered to $\leq$ grade 1 between days 32-45. No dose-limiting non-hematologic toxicities were observed.

A second phase I trial is being conducted at the MD Anderson Cancer Center in patients with advanced hematologic malignancies. Twenty-eight patients with relapsed or refractory leukemia (20

acute myeloid leukemia [AML], 3 myelodysplasia, 1 chronic myeloid leukemia in blast crisis, 3 acute lymphocytic leukemia, 1 chronic lymphocytic leukemia) have been accrued to the study at doses ranging from 220-708 mg/m^2. Through the dose of 708 mg/m^2, no dose-limiting non-hematologic toxicities were observed. At doses ≥ 400 mg/m^2, patients developed a transient infusion-related syndrome consisting of headache, nausea, vomiting, myalgias/cramps, facial flushing, dizziness, tachycardia, and hypotension. The infusion-related reaction was self-limited and resolved within several hours after completing treatment in all patients. Among the seven patients treated at 708 mg/m^2, one patient developed prolonged marrow aplasia (> 80 days) without evidence of leukemia. Thus, myelosuppression may be a dose-limiting toxicity (DLT) at 708 mg/m^2. An additional cohort of patients is currently being evaluated at an interim dose of 600 mg/m^2.

Evidence of anti-tumor activity was observed in patients with advanced hematologic malignancies. One previously untreated patient with high-risk myelodysplasia developed a complete response by day 28 after a single course of VNP40101M administered at 300 mg/m^2. Although no other patient achieved complete remission, VNP40101M reduced peripheral blood blasts at least transiently in most patients at all dose levels. In addition, a heavily pre-treated patient with AML had substantial clearing of marrow blasts and resolution of gingival leukemic infiltration at a dose of 220 mg/m^2, and a patient with AML treated at 532 mg/m^2 had reduction in marrow blasts and improvement of neutrophil counts by day 28. The level of activity warrants further exploration of VNP40101M alone and in combination in patients with AML.

SUMMARY OF THE INVENTION

This invention provides a method for treating tumor in a subject comprising administering to the subject an effective amount of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog. This invention also provides a method for inhibiting tumor cell growth comprising contacting the tumor cell with effective amounts of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog.

The present invention relates to the treatment of cancer, comprising administering to a subject in need thereof an effective amount of VNP40101M in combination with a nucleoside.

This invention provides a composition comprising an amount of VNP40101M which produces synergistic effects when used in combination with a nucleoside in treating tumor.

This invention provides a composition comprising an amount of nucleoside which produces synergistic effects when used in combination with a VNP40101M in treating tumor.

This invention provides a composition comprising an amount of an anticancer agent which produces synergistic effects when used in combination with a VNP40101M in treating tumor.

This invention provides other cancer therapies such as radiation or other chemotherapeutics which include, but are not limited to, antimetabolites, etoposide, doxorubicin, taxol, vincristine, cyclophosphamide, mitomycin C, topoisomerase I and topoisomerase II inhibitors (adriamycin, topotecan, campothecin and irinotecan), platinum containing compounds, (cisplatin, carboplatin), tipifarnib (R115777), SCH66336, erlotinib, gefitinib, and gemtuzumab ozogamicin, may be used with VNP40101M or its equivalent. VNP40101M or its equivalent provides synergistic effects when used in combination with these therapies.

DETAILED DESCRIPTION OF THE INVENTION

This invention provides a method for treating tumor in a subject comprising administering to the subject an effective amount of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog. This invention also provides a method for inhibiting tumor cell growth comprising contacting the tumor cell with effective amounts of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog.

VNP40101M - Cloretazine™

VNP40101M is a representative form of SHPs. As used herein, VNP40101M covers itself, its salt or other prodrug forms which produce same or similar effective *in vivo*. Prodrug is an inactive precursor which will be converted into its active form by normal metabolic processes.

VNP40101M and its equivalents are known in the art. See e.g. US Patent no. 6,855,695 B2, issued February 15, 2005. Other SHPs can also be used in the invention.

The present invention relates to the treatment of cancer, comprising administering to a subject in need thereof an effective amount of VNP40101M in combination with a nucleoside.

These agents may be administered concurrently or sequentially.

In an embodiment, the subjects are mammals. In a further embodiment, the subjects are human.

Nucleosides and their Analogs

As used herein, the nucleoside analog is defined as analog which produces substantially the same effect as the nucleoside itself. Nucleoside analogs are capable of inhibiting DNA synthesis or incorporating into DNA such as azacitidine, cladribine, decitabine. gemcitabine, mercaptopurine, thioguanine, fludarabine, clofarabine, troxacitabine, and pentostatin are useful to combine with VNP40101M to treat malignancies.

Cytarabine, a cell-cycle specific antimetabolite, is the most effective drug in the treatment of AML. Cytarabine is phosphorylated intracellularly and incorporated into DNA. By inhibiting DNA polymerases and DNA synthesis, cytarabine is predicted to inhibit DNA repair and enhance the cytotoxicity of VNP40101M.

Radiation or other Chemotherapeutics

Other cancer therapies such as radiation or other chemotherapeutics which include, but are not limited to, antimetabolites, etoposide, doxorubicin, taxol, vincristine, cyclophosphamide, mitomycin C, topoisomerase I and topoisomerase II inhibitors (adriamycin, topotecan, campothecin and irinotecan), platinum containing compounds, (cisplatin, carboplatin), tipifarnib (R115777), SCH66336, erlotinib, gefitinib, and gemtuzumab ozogamicin, may be used with VNP40101M or its equivalent. VNP40101M or its equivalent provides synergistic effects when used in combination with these therapies.

An aspect of the present invention relates to the treatment of cancer, comprising administering to a patient in need thereof an effective amount of VNP40101M in combination with at least one therapeutic agent. In an embodiment, the agent is a nucleoside or a nucleoside analog.

The treatment of solid malignant tumors, leukemia, and lymphomas is a preferred embodiment of the present invention. In a further embodiment, the cancer is acute myelogenous leukemia (AML).

The amount of VNP40101M and nucleoside or its analog used according to the present invention is an effective amount for treating cancer. In general, a therapeutically effective amount of the VNP40101M according to the present invention usually ranges from less than about 0.05 mg/kg to about 100 mg/kg of body weight of the patient to be treated, or considerably more, depending upon the compound used, the tumor type to be treated, the ability of the active compound to localize in the tissue to be treated, the route of administration and the pharmacokinetics of the compound in the patient.

VNP40101M is preferably administered in amounts ranging from about 0.5 mg/kg (17.5 mg/m^2) to about 50 mg/kg (1750 mg/m^2) or more at one time. Nucleoside or its analog is preferably administered in amounts ranging from about 0.1 mg/kg (3.5 mg/m^2) to about 150 mg/kg (5250 mg/m^2) or more at one time; preferably is given from 1 mg/kg to 100 mg/kg. The duration of treatment may be for one or more days or may last for several months or considerably longer (years) depending upon the disease state treated.

In a more preferred embodiment, VNP40101M is given to the patient at doses of 1 mg/kg to 20 mg/kg and the nucleoside at doses of 10 mg/kg to 80 mg/kg. In another preferred embodiment, the nucleoside is cytarabine (AraC) and the dose ranges from 20 mg/kg to 60 mg/kg. In a still further embodiment the does of VNP40101M is between 100 and 1000 mg/m^2 and the dose of AraC is between 500 and 5000 mg/m^2.

Synergistic Effects

This invention provides a composition comprising an amount of VNP40101M or its equivalent which produces synergistic effects when used in combination with a nucleoside or its analog in treating tumor.

This invention provides a composition comprising an amount of nucleoside which produces synergistic effects when used in combination with a VNP40101M in treating tumor.

As demonstrated in the below examples, this effective amount of either VNP40101M, its equivalent or nucleoside (its analog), can be routinely determined. These combinations of agents may be delivered intravenously, subcutaneously, intramuscularly, intraperitoneally or orally. Other routes of administration such as inhalation may also be used.

This invention will be better understood from the examples which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the

claims which follow thereafter.

EXPERIMENTAL DETAILS

Example 1. In vitro cytotoxicity of VNP40101M and AraC (cytarabine) on tumor cell lines

The cytotoxicity of the combination of VNP401 01M and Arac on L1210 leukemia was examined using a cell viability assay. Cells were exposed to VNP40101M, alone or in combination with various concentrations of AraC. After 72 hours, the remaining viable cells were quantified by measuring mitochondrial oxidoreductase activity. Concentrations of AraC and VNP401O1M used were between 0.75 and 6 uM. Dose-effect analyses (combination index) showing combination effects of VNP40101M and AraC were analyzed. Combination indices of 0.75 - 0.8 and 0.1 - 0.3 indicate moderate synergism and strong synergism, respectively (Chou and Talalay, *Adv. Enz. Regul.*, **22**, 27-55, 1984). Cytotoxic effect of VNP40101M was evaluated in the presence of AraC on L12l0 leukemia and table 1 shows that CLOETAZINE worked synergistically with AraC on leukemia.

Table 1: Combination of AraC and VNP40101M after 1 hour exposure to L1210 Cells.

AraC (μM)	VNP40101M (μM)	Fraction affected	Combination Index*
0.75	6	0.955	0.243
0.75	3	0.915	0.189
0.75	1.5	0.904	0.121
0.75	0.75	0.886	0.089
1.5	6	0.962	0.246
1.5	3	0.918	0.221
1.5	1.5	0.918	0.147
1.5	0.75	0.903	0.122
3	6	0.967	0.271
3	3	0.928	0.273
3	1.5	0.925	0.209
3	0.75	0.924	0.175
6	6	0.958	0.405
6	3	0.943	0.357
6	1.5	0.933	0.325
6	0.75	0.925	0.312

Example 2. In vitro cytotoxicity of VNP40101M and Fludarabine on tumor cell lines

The cytotoxicity of the combination of VNP40101M and nucleoside analogs on tumor cell lines is examined using a cell viability assay. Several leukemia cell lines (L1210 and HL-60) and lymphoma cell lines (Raji and Namalwa) are exposed to VNP40101M, alone or in combination with various concentrations of Fludarabine. After 72 hours, the remaining viable cells were quantified by measuring mitochondrial oxidoreductase activity. Concentrations of Fludarabine used are between 0.1 to 100 uM; concentrations of VNP40101M used are between 0.1 to 100 uM. Dose-effect analyses (combination index) showing combination effects of VNP40101M and Fludarabine are analyzed. Combination indices of 0.75 - 0.8 and 0.1 - 0.3 indicate moderate synergism and strong synergism, respectively.

Example 3. In vivo antitumor activity of VNP40101M and AraC on leukemia

Eighty Female Balb/c x DBA/2 (CD_2F1) mice were inoculated intraperitoneally (ip) on day 0 with $1x10^6$ L1210 cells in 0.2mL phosphate-buffered-saline (PBS). The mice were randomly divided into 6 groups; each group consisted of 10 mice. The animals were untreated or treated with a single bolus dose of VNP40101M at 5, or 10mg/kg, ip, on day 1 with or without AraC (50mg/kg, ip) on days 1, 3, 5, 7, and 9. During the experiments, mice were observed daily for survival. It was determined that mice that survive for more than 60 days after inoculation of L1210 cells might be regarded as long-term survivors. Kaplan-Meier plots were generated, and survival time of animals was analyzed using student T-test. Significance was defined as $P < 0.05$.

Table 2 shows that intraperitoneal inoculation of mice with $1x10^6$ L1210 leukemia cells resulted in a rapid development of ascites followed by death of all animals in untreated control within 18 days and in AraC treatment control within 30 day. VNP40101M treatment at single doses of 5 and 10mg/kg increased long-term survivors to 50% and 90%, respectively. Therapeutic

efficacies of combinational treatments were superior to single agents. VNP40101M, at 5 and 10mg/kg, plus AraC treatments increased long-term survivors to 80% and 100%, respectively. Our results suggest that VNP40101M significantly enhanced anti-tumor effect of AraC.

Table 2. Therapeutic efficacy of single agents vs. combinations

Group	Long-term Survivors (%)	P value*
1. Untreated Control	0	
2. AraC 50mpk	0	
3. VNP40101M 5mpk	50	
4. VNP40101M 10mpk	90	
5. AraC 50mpk+VNP40101M 5mpk	80	0.0024
6. AraC 50mpk +VNP40101M 10 mpk	100	0.0022

*Combinational therapy against VNP40101M at corresponding doses

Example 4. In vivo antitumor activity of VNP40101M and fludarabine on leukemia

Eighty Female Balb/c x DBA/2 (CD_2F1) mice are inoculated intraperitoneally (ip) on day 0 with 3 $x10^6$ L1210 cells in 0.2mL phosphate-buffered-saline (PBS). The mice are randomly divided into 6 groups; each group consists of 10 mice. The animals are untreated or treated with a single bolus dose of VNP40101M at 5, or 10mg/kg, ip, on day 1 with or without fludarabine (70 mg/kg, ip) on multiple days between days 1 to 9. During the experiments, mice are observed daily for survival. It is determined that mice that survive for more than 60 days after inoculation of L1210 cells might be regarded as long-term survivors. Kaplan-Meier plots are generated, and survival time of animals is analyzed using student T-test. Significance is defined as $P < 0.05$.

Table 3 shows that intraperitoneal inoculation of mice with $3x10^6$ L1210 leukemia cells resulted in a rapid development of ascites followed by death of all animals in untreated control within 18 days and in fludarabine treatment control within 30 day. VNP40101M treatment at single doses 10mg/kg increased long-term survivors to 40%. Therapeutic efficacy of combinational treatment was superior

to single agents. VNP40101M, at 10mg/kg plus fludarabine treatments increased long-term survivors to 90%. Our results suggest that VNP40101M significantly enhanced anti-tumor effect of fludarabine.

Table 3. Therapeutic efficacy of single agents vs. combinations

Group	Long-term Survivors (%)	P value*
1. Untreated Control	0	
2. Fluda 70mpk	0	
3. VNP40101M 5mpk	0	
4. VNP40101M 10mpk	40	
5. Fluda 70mpk+VNP40101M 5mpk	0	
6. Fluda 70mpk +VNP40101M 10 mpk	90	0.0025

Example 5. Use of VNP40101M and AraC in patients with AML

AraC is administered by IV continuous infusion at a dose of 0.5 to 4.0 gm/m^2/day for one to six days. Preferably, AraC is administered at a dose of 1.0 to 2.0 gm/m^2/day for three to four days. VNP40101M is administered over 15-30 minutes on day 1 to day 3 after AraC infusion. The dose of VNP40101M is between 200 to 700 mg/m^2. Preferably, VNP40101M is administered one day after AraC infusion and the dose is between 450 to 650 mg/m^2. Multiple cycles of treatment can be repeated.

Example 6. Use of VNP40101M and clofarabine in patients with AML

Clofarabine is administered by IV at a dose of 10 to 50 mg/m^2/day for one to six days. Preferably, clofarabine is administered at a dose of 30 to 40 mg/m^2/day for five consecutive days. VNP40101M is administered over 15-60 minutes as a single bolus injection before, during, or after clofarabine administration. The dose of VNP40101M is between 200 and 700 mg/m^2. Preferably, VNP40101M dose is between 450 and 650 mg/m^2. Multiple cycles of treatment can be repeated.

Example 7. Use of VNP40101M and tipifarnib in patients with AML

Tipifarnib is administered orally at 200 to 800 mg/dose, one to three doses a day for up to 28 days. Preferably, tipifarnib is administered at 600 mg/dose, two doses per day, for 28 consecutive days. VNP40101M is administered over 15-60 minutes as a single bolus injection before, during, or after administration of tipifarnib. The dose of VNP40101M is between 200 to 700 mg/m^2. Preferably, VNP40101M is between 450 and 650 mg/m^2. Multiple cycles of treatment can be repeated.

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What is claimed is:

1. A method for treating tumor in a subject comprising administering to the subject an effective amount of:

(a) VNP40101M, or its equivalent; and

(b) a nucleoside, or a nucleoside analog.

2. A method for inhibiting tumor cell growth comprising contacting the tumor cell with effective amounts of:

(a) VNP40101M, or its equivalent; and

(b) a nucleoside, or a nucleoside analog.

3. The method of claim 1 or 2 wherein the nucleoside is AraC (cytarabine), azacitidine, cladribine, decitabine, gemcitabine, mercaptopurine, thioguanine, fludarabine, clofarabine, troxacitabine or pentostatin.

4. The method of claim 1 or 2, wherein the tumor is solid malignant tumor, leukemia or lymphoma.

5. The method of claim 1 or 2, wherein VNP40101M and the nucleoside can be administered concurrently or sequentially.

6. The method of claim 1, wherein VNP40101M and the nucleoside are administered intravenously, subcutaneously, or orally.

7. A method for treating cancer comprising administering to a subject in need thereof an effective amount of VNP401OM and a nucleoside.

8. The method of claim 7, wherein the cancer is a leukemia and the nucleoside is AraC.

9. The method of claim 7, wherein the cancer is a leukemia and the nucleoside is clofarabine.

10. The method of claim 8 or 9, wherein the leukemia is acute myelogenous leukemia.

11. The method of claim 8, wherein the dose of VNP40101M is between 100 and 1000 mg/m^2 and the dose of AraC is between 500 and 5000 mg/m^2.

12. A method for treating tumor in a subject comprising administering to the subject:

(a) an effective amount of VNP40101M, or its equivalent; and

(b) another anti-tumor therapy.

13. A method for inhibiting tumor cell growth comprising contacting the tumor cell:

(a) with effective amounts of VNP40101M, or its equivalent; and

(b) another anti-tumor therapy.

14. The method of claim 12 or 13 wherein the other anti-tumor therapy is radiation therapy or administration of another chemotherapeutic agent.

15. The method of claim 14 wherein the chemotherapeutic agent is antimetabolites, etoposide, doxorubicin, taxol, vincristine, cyclophosphamide, mitomycin C, topoisomerase I and topoisomerase II inhibitors (adriamycin, topotecan, campothecin and irinotecan), cisplatin, carboplatin, tipifarnib (R115777), SCH66336, erlotinib, gefitinib, or gemtuzumab ozogamicin.

16. The method of any of claim 1-2, 6-12 and 14-15 wherein the subject is a human.

17. A composition comprising an amount of VNP40101M which produces synergistic effects when used in combination with a nucleoside in treating tumor.

18. A composition comprising an amount of nucleoside which produces synergistic effects when used in combination with a VNP40101M in treating tumor.

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
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(54) Title: COMBINATION THERAPY COMPRISING CLORETAZINE™

(57) Abstract: This invention provides a method for treating tumor in a subject comprising administering to the subject an effective amount of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog. This invention also provides a method for inhibiting tumor cell growth comprising contacting the tumor cell with effective amounts of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog. The present invention relates to the treatment of cancer, comprising administering to a subject in need thereof an effective amount of VNP40101M in combination with a nucleoside.

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AMENDED CLAIMS
[received by the International Bureau on 19 May 2006 (19/05/2005)
Claims 19-33 added]

19. A method of treating tumor growth in a subject comprising administering
(a) VNP40101M, or its equivalent; and
(b) a nucleoside, or a nucleoside analog,

wherein the amounts of (a) and (b) administered provide a reduction in tumor growth that is larger than that achieved by administering either (a) or (b) individually.

20. A method of treating tumor growth in a subject comprising administering components

(a) VNP40101M; and
(b) a nucleoside, or a nucleoside analog,

wherein the amounts of (a) and (b) administered provide a reduction in tumor growth that is larger than that achieved by administering either (a) or (b) individually.

21. A method of treating tumor growth in a subject comprising administering

(a) VNP40101M, or its equivalent; and
(b) a nucleoside, or a nucleoside analog is selected from the group consisting of AraC (cytarabine), azacitidine, cladribine, decitabine, gemcitabine, mercaptopurine, thioguanine, fludarabine, clofarabine, troxacitabine and pentostatin.

22. The method of claim 21, comprising administering VNP40101M.

23. A method of obtaining a reduction in tumor size or tumor growth in a subject comprising administering a composition comprising

(a) VNP40101M, or its equivalent; and
(b) a nucleoside, or a nucleoside analog selected from the group consisting of AraC (cytarabine), azacitidine, cladribine, decitabine, gemcitabine, mercaptopurine, thioguanine, fludarabine, clofarabine, troxacitabine and pentostatin.

24. A method of obtaining a reduction in tumor size growth in a subject comprising administering

(a) VNP40101M; and
(b) either fludarabine or cytarabine.

25. A method for inhibiting tumor growth in a subject comprising administering to the subject an effective amount of:

(a) VNP40101M, or its equivalent; and
(b) a nucleoside, or a nucleoside analog

and wherein the effective inhibiting of tumor growth of (a) and (b) is approximately additive, or greater than additive.

26. A method for treating a tumor in a subject comprising the step of administering to the subject an effective amount of a composition comprising:

(a) VNP40101M, or its equivalent; and
(b) a nucleoside, or a nucleoside analog,

and, wherein (a) does not diminish the efficacy of (b) nor does (b) diminish the efficacy of (a).

27. The method of claim 21 or claims 23-26 wherein the tumor is a solid tumor, a lymphoma or leukemia.

28. A composition comprising
(a) VNP40101M, or its equivalent; and
(b) a nucleoside, or a nucleoside analog

and a pharmaceutically acceptable carrier, adjuvant or filler.

29. The composition of claim 28 wherein the nucleoside or nucleoside analog is selected from the group consisting of AraC (cytarabine), azacitidine, cladribine, decitabine, gemcitabine, mercaptopurine, thioguanine, fludarabine, clofarabine, troxacitabine and pentostatin.

30. The composition of claim 29 wherein the nucleoside or nucleoside analog is either cytarabine or fludarabine.

31. The composition of claim 28 comprising VNP40101M and either cytarabine or fludarabine.

32. The composition of claim 28 comprising VNP40101M and cytarabine.

33. The composition of claim 28 comprising VNP40101M and fludarabine

COMBINATION THERAPY COMPRISING CLORETAZINE™

ABSTRACT OF THE INVENTION

This invention provides a method for treating tumor in a subject comprising administering to the subject an effective amount of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog. This invention also provides a method for inhibiting tumor cell growth comprising contacting the tumor cell with effective amounts of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog. The present invention relates to the treatment of cancer, comprising administering to a subject in need thereof an effective amount of VNP40101M in combination with a nucleoside.

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(74) Agent: **CHAN, Albert, Wai-Kit**; World Plaza, Suite 604, 141-07 20th Avenue, Whitestone, NY 11357 (US).

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(54) Title: COMBINATION THERAPY COMPRISING CLORETAZINE™

(57) **Abstract:** This invention provides a method for treating tumor in a subject comprising administering to the subject an effective amount of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog. This invention also provides a method for inhibiting tumor cell growth comprising contacting the tumor cell with effective amounts of: (1) VNP40101M, or its equivalent; and (2) a nucleoside, or a nucleoside analog. The present invention relates to the treatment of cancer, comprising administering to a subject in need thereof an effective amount of VNP40101M in combination with a nucleoside.

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INTERNATIONAL SEARCH REPORT

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P --- Y	US 6,855,695 B2 (LIN et al) 15 February 2005 (15.02.2005), entire document.	1-18
Y	LEE et al. Toxicological evaluation of 1,2 bis(methylsulfonyl)-1-(2-chloroethyl)-2-(methylaminocarbonyl) hydrazine (VNP40101M), novel alkylating Agent with Potential Antitumor Activity, with Intravenous Administration in Rats and Dogs. Vol. 21, pp. 23-28, 2002.	1-18

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2005 (30.10.2005)	21 MAR 2006
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer Howard V. Owens Telephone No. 7033081235

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INTERNATIONAL SEARCH REPORT

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[54] 发明名称

包含 Cloretazine™ 的治疗组合物

[57] 摘要

本发明提供了一种治疗患者肿瘤的方法，其包含给患者施用有效量的：(1) VNP40101M，或其等价物；和(2)核苷，或核苷类似物。本发明还提供了一种抑制肿瘤细胞生长的方法，包括将肿瘤细胞接触有效量的：(1) VNP40101M，或其等价物；和(2)核苷，或核苷类似物。本发明涉及癌症的治疗，包含给患者施用其所需的有效量的与核苷组合的 VNP40101M。

1、一种治疗患者肿瘤的方法，其包含给患者施用有效量的：
（a）VNP40101M，或其等价物；和（b）核苷，或核苷类似物。

2、一种抑制肿瘤细胞生长的方法，其包含将肿瘤细胞接触有效量的：
（a）VNP40101M，或其等价物；和（b）核苷，或核苷类似物。

3、根据权利要求1或2的方法，其中核苷为AraC（阿糖胞苷）、氮胞苷、克拉屈滨（cladribine）、地西他滨（decitabine）、吉西他滨（gemcitabine）、颈基嘌呤、硫鸟嘌呤、氟达拉宾（fludarabine）、氯法拉滨（clofarabine）、troxacitabine或戊糖苷。

4、根据权利要求1或2的方法，其中肿瘤是固体恶性肿瘤、白血病或淋巴瘤。

5、根据权利要求1或2的方法，其中VNP40101M和核苷可以同时或连续给药。

6、根据权利要求1的方法，其中VNP40101M和核苷静脉注射、皮下或口服给药。

7、一种治疗癌症的方法，其包含给患者施用其所需的有效量的VNP4010M和核苷。

8、根据权利要求7的方法，其中癌症是白血病和核苷是AraC。

9、根据权利要求7的方法，其中癌症是白血病和核苷是氯法拉滨。

10、根据权利要求8或9的方法，其中白血病是急性骨髓白血病。

11、根据权利要求8的方法，其中VNP40101M的剂量为100-1000mg/m^2和AraC的剂量为500-5000mg/m^2。

12、一种治疗患者肿瘤的方法，其包含给患者施用：
（a）有效量的VNP40101M，或其等价物；和（b）其它抗肿瘤治疗。

13、一种抑制肿瘤生长的方法，其包含将肿瘤细胞接触：
（a）有效量的VNP40101M，或其等价物；和（b）其它抗肿瘤治疗。

14、根据权利要求12或13的方法，其中其它的抗肿瘤治疗是放射治疗或施用其它化疗药剂。

15、根据权利要求14的方法，其中化疗药剂是抗代谢物、依托泊苷、阿

霉素、紫杉酚、长春新碱、环磷酰胺、丝裂霉素 C、拓扑异构酶 I 和拓扑异构酶 II 抑制剂（阿霉素、拓扑替康（topotecan）、喜树碱（campothecin）和药薯）、顺氯氨铂、卡铂（carboplatin）、替匹法尼（tipifarnib）(R115777)、SCH66336、埃罗替尼（erlotinib）、吉非替尼片（gefitinib）、或吉妥单抗（gemtuzumab ozogamicin）。

16、根据权利要求 1-2、6-12 和 14-15 任一项的方法，其中患者是人。

17、一种组合物，其包含一定量的与核苷组合使用治疗肿瘤时能够产生协同作用的 VNP40101M。

18、一种组合物，其包含一定量的与VNP40101M组合使用治疗肿瘤时能够产生协同作用的核苷。

包含 Cloretazine™ 的治疗组合物

本申请受益于2004年3月26日申请的美国系列申请号:60/556,565,将前述申请在此全部作为参考并入本申请。

贯穿本申请,参考了各种出版物。这些出版物的全部公开内容在此作为参考并入本申请以更全面地描述本申请所属领域的技术状态。

技术领域

本发明涉及包含 Cloretazine™ 的治疗组合物。本发明还涉及 Cloretazine™ 和核苷的协同作用。

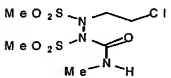
背景技术

烷化剂是目前用于治疗各种恶性肿瘤的最有效的药物之一,并且被广泛用于临床(Katzung, 基础及临床药理学,第七版,1998, Appleton & Lange, Stamford, 881),高细胞毒性是由于其具有导致 DNA 链间交联因而抑制复制的能力(Rajski a 和 Williams, 化学评论,1998, 98:2723)。在烷化剂中,CNU(氯乙基亚硝基脲)族已经被广泛地应用于临床治疗脑肿瘤、结肠癌和淋巴瘤(DeVita 等,癌症研究,1965,25:1876;和 Nissen 等,癌症,1979, 43:31),然而,由于迟发的和累积的骨髓机能降低和肝毒性限制了它们的临床应用(Panasci 等,癌症研究,1977, 37:2615;和, Gibson 与 Hickman, 生化药学,1982, 31:2795)。

由 CNUs 产生的许多具有生成氯乙基化和甲氨酰化类但不能生成羟乙基化和乙烯化类的 1,2-二(磺酰基)肼的药物前体(SHPs)最近被开发了出来(Sartorelli 等,参见美国专利号: 6,040,338;美国专利号: 5,637,619; 美国专利号:5,256,820; 美国专利号:5,214,068; 美国专利号:5,101,072;美国专利号:4,849,563;和美国专利号:4,684,747)。抗肿瘤活性也已经提出是由 DNA 的氯乙基化和后交联产生(Kohn,癌症研究的最近成果,Eds. Carter 等,1981,Springer,Berlin,

vol.76:141；和 Shealy 等，药物化学期刊，1984， 27:664)。甲氨酰化类（也就是异氰酸酯）能够与蛋白质上的巯基和胺官能团反应并抑制 DNA 聚合酶（Baril 等，癌症研究，1975，35: 1)、DNA 链断裂的修复（Kann 等，癌症研究，1974，34:398）和 RNA 合成及其加工（Kann 等，癌症研究，1974，34:1982)。无论如何，DNA 的羟乙基化是致癌的和/或诱变的事件（Swenson 等，J Natl Cancer Inst.，1979，63:1469)。

1,2-二（甲磺酰基）-1-(2-氯乙基)-2-(甲氨基羰基)肼（VNP40101M，Cloretazine™)，SHP 族中的最新的前导化合物，对宿主具有低毒性，而且与氯乙基亚硝基脲（CNU）衍生物和其它 SHP 类似物相比，对 L1210 鼠白血病、L1210/BCNU、L1210/CTX、L1210/MEL (1,3-二（2-氯乙基）-1-亚硝基脲，环磷酰胺和苯丙氨酸抗性亚系)、P388 白血病、M109 肺癌、B16 黑素瘤、C26 结肠癌和 U251 神经胶质瘤具有更好的抗肿瘤活性(Shyam 等，药物化学期刊，1999，42:941)。另外，VNP40101M 在越过脑血管屏障（BBB）和根除颅内植入的白血细胞（>6.54 对数细胞杀死量)、对抗 BCNU 效力是有效果的(Finch 等，癌症生化生物物理学，2001,61: 3033)。



VNP40101M

VNP40101M 的抗肿瘤活性可能归因于 90CE 和甲基异氰酸酯的释放。90CE 进一步分裂产生甲基 2-氯乙基重氮砜（1)，参考美国专利号 6,855,695 中的图 1，相对特异性的 O^6-鸟嘌呤氯乙基因子（chloroethylator)，鸟嘌呤 N^7位少量的烷基化产生（Penketh 等，药物化学期刊，1994，37:2912；和 Penketh 等，生化药学，2000，59: 283)。由 VNP40101M 释放的甲基异氰酸酯具有抑制各种 DNA 修复酶的能力，这些酶包括使 DNA 中 O^6-烷基鸟嘌呤单烷基类稳定的 O^6-烷基鸟嘌呤-DNA 烷基转移酶（Baril 等， 癌症研究，1975，35: 1)。

在鼠肿瘤模型中的活性

VNP40101M 已经显示了对模型鼠中的白血病和固体同源及人异种移植肿瘤的广泛的抗肿瘤活性（Shyam 等，药物化学期刊，28:525-7，1985；Shyam 等，药物化学期刊，29:1323-5， 1986；Shyam 等，药物化学期刊，30:2157-61，1987；Shyam 等，药物化学期刊，36:3496-502，1993；Shyam 等，药物化学期刊，39:796-801，1996）。资料简要概括如下：

1. 抗腹膜内（IP）植入的 L1210 白血病（10^6 肿瘤细胞），该白血病对 1,3-二（2-氯乙基）-1-亚硝基脲（BCNU）、环磷酰胺、或苯丙氨酸具有抗性，肿瘤接种（20-60mg/kg，或 60-180mg/m^2）后 24 小时单次剂量腹膜内施药 VNP40101M 对小鼠具有 100%治愈（生存期≥60 天）。能够在敏感和抗性的具有 L1210 的小鼠上产生长期存活的 VNP40101M 剂量当施药于非肿瘤的小鼠中时仅仅是适当的骨髓抑制。一些动物施用更高的单次剂量（80-100mg/kg）治疗多于 50 天后产生消瘦状态体征和死亡。当连续 6 天每天腹膜内施用 6mg/kg/d 剂量的 VNP40101M（总剂量为 36mg/kg）治疗的小鼠相对于标准小鼠增加了 333％的生存期限，而施用剂量为 12-18mg/kg/d（总剂量为 72-108mg/kg）对动物的治愈为 100%。用 d×6 日程表并没有发现延迟的毒性死亡，但是总剂量高于 108mg/kg 的情况并没有试验。
2. 抗裸鼠内的约 300mg 的人 U251 神经胶质瘤的皮下肿瘤，以 10mg/kg q2d×11 或 20mg/kg q4d×6 腹膜内施药 VNP40101M 导致肿瘤完全的消退。
3. 抗皮下种植的约 150mg 的鼠 M109 肺癌，以 10 mg/kg q2d×10 或 20 mg/kg q4d×5 腹膜内施药 VNP40101M 延迟了肿瘤生长，用后一个日程表经 14 天延迟时间使肿瘤达到 1g。
4. 通过腹膜内每周给药 VNP40101M 来抗同源 B16F10 黑素瘤、人 HTB177 肺（H460）和 WiDr 结肠癌细胞系产生显著的肿瘤生长延迟，10－60 mg/kg 的剂量是有效的，但是高剂量产生更强的生长抑制。
5. 通过腹膜内给药抗颅内植入的 L1210 白血病发现了 VNP40101M 的皮下抗肿瘤活性，证明其通过血脑屏障的良好穿透能力。

毒性研究

在正常的 CD2F1 小鼠内检验了 VNP40101M 剂量和白血细胞量（WBC）之间的关系。用单次腹膜内施药剂量 40 mg/kg (120 mg/m^2)发现了最适当的白血球减少症（基准的 50%）。完全恢复的情况下，60-80 mg/kg 剂量的 VNP40101M 施药 4 天和 21 天分别减少 WBC 的量到基准的约 25%和 15%。如在 1.2.A 部分所述，80-100 mg/kg 的高单次剂量腹膜内施药给具有肿瘤的小鼠，在治疗存活 50 天后产生消瘦体征和死亡。

在大鼠体内进行的毒性研究。根据 d×5 剂量日程表静脉注射（IV）给药 3 mg/kg(18mg/m^2)，在 15 天的时候没有产生临床体征和征状，但是在 29 天的时候 2/10 的大鼠具有肺部发现，包括少量的胸腔液体和肺衰竭至塌陷。在 3 mg/kg (d×5)剂量水平的显微发现主要限于肺部，包括肺泡水肿、充血、肺泡组织细胞增多病，和血管血栓。10 mg/kg(60 mg/m^2) d×5 的高剂量在 15 天时产生很少显著的肉眼体检发现，但是 29 天的时候在大约 50%的动物胸腔发现液体和在 30、31 天分别有 6 只动物死亡。肺部组织病理学的发现与 3mg/kg 剂量水平相似。在与 10mg/kg d×5 一样高的剂量下，没有发现脊髓压迫。血清化学的影响仅限于总蛋白和清蛋白的减少，在 10 mg/kg 剂量组的第 29 天发现该影响。

在狗体内进行的毒性研究，以 1、3、10 和 30mg/kg 的单次剂量静脉注射施药。1 和 3 mg/kg 具有很好的耐受性并且在至少 21 天才发现产生了微小的临床体征。10 和 30mg/kg (分别为 200 和 600mg/m^2)的高剂量产生了显著的临床体征，还有实验室异常，其包括碱性磷酸酶增加、白蛋白降低、胆红素增加、肌酸磷酸激酶增加、和白血细胞、红血细胞量降低。还确定了 d×5 静脉注射 0.3、1 和 3mg/kg 的剂量的毒性。3 mg/kg d×5 的剂量产生了显著的临床体征，包括活动减少、大便失禁、厌食和轻微的脱水，8 天的时候有动物开始死亡。经过 8 天还表现了显著的白细胞减少和碱性磷酸酯酶轻微升高。1mg/kg(20mg/m^2)d×5 的剂量在 8 天的时候产生微小的临床体征和征状，和仅有的轻微的白细胞减少而且经过 15 天恢复到基准值。

VNP40101M 的 I 阶段研究

在具有晚期固体肿瘤或血液学恶性肿瘤的患者中实施了 VNP40101M

的两次 I 期试验研究。在第一次 I 期试验中，26 个具有晚期固体肿瘤的患者接受了 15－30 分钟以上的静脉注射，剂量水平为每 4-6 周 3-305mg/m²。最大耐受剂量（MTD）为 305mg/m²。在以最大耐受剂量治疗的 7 名患者中，6 名患者血小板减少到 3 级。血小板最低点发生在 25-33 天。以最大耐受剂量治疗患者中的 5 名出现血小板减少大于等于 2 级，但是仅有 1 名患者为 3 级。血液学毒性在第 32-45 天的时候恢复到小于等于 1 级。观察到了没有剂量限制的非血液学毒性。

第二次 I 期试验是在 MD 安德森肿瘤中心（MD Anderson Cancer Center）对具有晚期血液学恶性肿瘤的患者实施的。28 名具有复发或顽固性白血病患者（20 名急性骨髓白血病（AML）、3 名骨髓发育不良，1 名处于胚细胞转变期的慢性骨髓白血病，3 名急性淋巴细胞白血病，1 名淋巴细胞白血病）接受了剂量为 220-708mg/m² 的治疗研究。虽然发现了 708 mg/m² 剂量、非剂量限制的非血液学毒性。但在剂量大于等于 400mg/m² 时，患者出现了短暂的输注相关综合症，包括头疼、恶心、呕吐、肌疼/痛性痉挛，面部发红、头晕、心动过速、和低血压。输注相关的反应具有自身局限性并且所有的患者在其完成治疗的几个小时后都消退了。在以 708mg/m² 治疗的 7 名患者中，1 名患者出现长时间骨髓发育不全（>80 天）且没有白血病迹象。因此，在 708mg/m² 剂量骨髓抑制可能是一种剂量限制的毒性（DLT）。另外一批患者目前正在以 600mg/m² 的过渡剂量进行评价。

在晚期血液学肿瘤患者中发现了抗肿瘤的证据。一位早期未治疗的高危脊髓发育不良患者在单次剂量施药 300mg/m² 的 VNP40101 后经 28 天发生完全性反应。而没有其它的患者获得完全的缓解，在所有的剂量水平，大部分患者至少暂时通过 VNP40101M 减少了外周血胚细胞。另外，治疗前严重的 AML 患者通过 220mg/m² 的剂量基本了清除了骨髓胚细胞和消散了齿龈白血病浸润，和以 532 mg/m² 剂量治疗的 AML 患者经过 28 天已经减少了骨髓胚细胞和提高了嗜中性白细胞的量。单独的和组合的 VNP40101M 的活性水平保证在 AML 患者中对其进一步探测。

发明内容

本发明的目的是：

提供一种治疗患者肿瘤的方法，包括给患者施用有效量的：

（1）VNP40101M，或其等价物；和（2）核苷，或核苷类似物。

本发明还提供一种抑制肿瘤细胞生长的方法，包括将肿瘤细胞接触有效量的：（1）VNP40101M，或其等价物；和（2）核苷，或核苷类似物。

本发明涉及癌症的治疗，包括给患者施用其所需的有效量的VNP40101M与核苷的组合物。

本发明提供了一种组合物，其包含相当数量的与核苷组合使用治疗肿瘤时产生增效作用的VNP40101M。

本发明提供了一种组合物，其包含相当量的与 VNP40101M 组合使用治疗肿瘤时产生增效作用的核苷。

本发明提供了一种组合物，其包含相当量的与 VNP40101M 组合使用治疗肿瘤时产生增效作用的抗癌药剂。

本发明提供了其它的癌症治疗方法，如放射疗法和其它的化学疗法，其包含但不限于：抗代谢物、依托泊苷、阿霉素、紫杉酚、长春新碱、环磷酰胺、丝裂霉素 C、拓扑异构酶 I、拓扑异构酶 II 抑制剂（阿霉素、拓扑替康（topotecan）、喜树碱（campothecin）和药薯）、含铂化合物（顺氯氨铂、卡铂（carboplatin））、替匹法尼（tipifarnib）(R115777)、SCH66336、埃罗替尼（erlotinib）、吉非替尼片（gefitinib）、和吉妥单抗（gemtuzumab ozogamicin），它们可以与 VNP40101M 或其等价物一起使用。当 VNP40101M 与这些疗法结合使用时提供增效作用。

本发明的目的具体可以通过以下措施达到：

本发明提供了一种治疗患者肿瘤的方法，包括给患者施用有效量的：

（1）VNP40101M，或其等价物；和（2）核苷，或核苷类似物。

本发明还提供一种抑制肿瘤细胞生长方法，包括将肿瘤细胞接触有效量的：（1）VNP40101M，或其等价物；和（2）核苷，或核苷类似物。

VNP40101M - Cloretazine™

VNP40101M 是 SHPs 中的一种代表形式。如本文所用的，VNP40101M 包含其本身、它的盐或在体内能够产生相同或相似作用其它的前体药物

形式。前体药物是通过正常代谢过程能够转化成其活性形式的非活性前身。

VNP40101M 和其等价物在本领域是已知的。参考如美国专利号：6,855,695 B2，发行于 2005 年 2 月 15 日。其它的 SHPs 也可以用于本发明。

本发明涉及癌症的治疗，包括给患者施用其所需的有效量的与核苷组合的 VNP40101M。

这些药剂可以同时或按顺序给药。

在一个实施方案中，患者是动物。在另一实施方案中，患者是人。

核苷及其类似物

如本文所用，核苷类似物被定义为能够与核苷本身产生基本相同效果的类似物。核苷类似物能够抑制 DNA 合成或向 DNA 中的插入，如将氮胞苷、克拉屈滨（cladribine）、地西他滨（decitabine）、吉西他滨（gemcitabine）、颈基嘌呤、硫鸟嘌呤、氟达拉宾（fludarabine）、氯法拉滨（clofarabine）、troxacitabine、和戊糖苷用于与 VNP40101M 结合治疗恶性肿瘤。

阿糖胞苷，细胞周期特异性抗代谢产物，是治疗 AML 的最有效的药物。阿糖胞苷被胞内磷酸化且被结合到 DNA 中。通过抑制 DNA 聚合酶和 DNA 合成，阿糖胞苷被预言抑制 DNA 修复和增强 VNP40101M 的细胞毒性。

放疗和其它化学疗法

其它的癌症治疗如放疗或其它的化疗包括但不限于抗代谢产物、依托泊苷、阿霉素、紫杉酚、长春新碱、环磷酰胺、丝裂霉素 C、拓扑异构酶 I、拓扑异构酶 II 抑制剂（阿霉素、拓扑替康、喜树碱和药薯）、含铂化合物（顺氯氨铂、卡铂）、替匹法尼（R115777）、SCH66336、埃罗替尼、吉非替尼片、和吉妥单抗，它们可以与 VNP40101M 或其等价物一起使用。当 VNP40101M 与这些疗法结合使用时提供增效作用。

本发明的一方面涉及癌症的治疗，包含给患者施用所需的有效量的与至少一种治疗药剂组合的 VNP40101M。在一个实施方案中，药剂是核苷或核苷类似物。

本发明优选的实施方案为固体恶性肿瘤、白血病、淋巴瘤的治疗。

在另一个实施方案中，癌症是急性骨髓白血病（AML）。

本发明中所用的 VNP40101M 和核苷或其类似物的量为治疗癌症的有效量。一般，根据所用的化合物、治疗的肿瘤类型、活性化合物在治疗组织中的定位能力、施药方式和化合物在患者中的药物动力学，本发明中的 VNP40101M 有治疗效果的量为小于治疗患者体重的约 0.05mg/kg 至约 100mg/kg，或更多。

VNP40101M 的优选给药量为一次约 0.5mg/kg(17.5mg/m²) 至约 50mg/kg(1750 mg/m²)或更多。核苷或其类似物优选给药量为一次约 0.1 mg/kg(3.5mg/m²) 至约 150 mg/kg(5250mg/m²) 或更多，优选给药量为 1mg/kg 至 100mg/kg。根据治疗疾病的状态，治疗持续的时间可以为 1 天或几天或几个月或更长（几年）。

在更加优选的实施方案中，VNP40101M 是以 1mg/kg 至 20mg/kg 的剂量向患者给药，而核苷是 10mg/kg 至 80mg/kg。在另一个优选的实施方案中，核苷是阿糖胞苷（Arac）且剂量是 20mg/kg 至 60mg/kg。还有，在另一个实施方案中，VNP40101M 的剂量为 100-1000mg/m²，同时 AraC 的剂量为 500-5000mg/m²。

协同作用

本发明提供了一种包含一定量的 VNP40101M 或其等价物的组合物，VNP40101M 或其等价物与核苷或其类似物组合使用治疗肿瘤时能够产生协同作用。

本发明提供了一种包含一定量的核苷或其类似物的组合物，核苷或其类似物与 VNP40101M 组合使用治疗肿瘤时能够产生协同作用。

如下面的实施例的证明，VNP40101M、其等价物或核苷（它的类似物）的有效量能够常规测定。这些药剂的组合可以静脉、皮下、肌内、腹膜内或口服递送。其它的给药方式如吸入也可以应用。

本发明将通过下面的实施例更好地得到理解，无论如何，本领域技术人员将很容易地领会到讨论的特定的方法和结果仅仅是本发明的解释，其在下面的权利要求中被更全面地描述。

本发明的优点是：

对宿主具有低毒性，由 VNP40101M 释放的甲基异氰酸酯具有抑制各种 DNA

修复酶的能力，具有更好的抗肿瘤活性。

另外，VNP40101M 在越过脑血管屏障（BBB）和根除颅内植入的白血细胞（>6.54 对数细胞杀死量）、对抗 BCNU 效力是有效果的。

具体实施方式

实施例 1、VNP40101M 和 AraC（阿糖胞苷）对体外肿瘤细胞系的细胞毒性

通过细胞活性分析测定了 VNP40101M 和 AraC 的组合物对 L1210 白血病的细胞毒性。将细胞暴露于单独的或与各种浓度 AraC 组合的 VNP40101M。72 小时后，通过测量线粒体 RNA 氧化还原酶活性来定量剩余的存活的细胞数量。所用的 AraC 和 VNP40101M 的浓度为 0.75-6μM。对显示 AraC 和 VNP40101M 联合效果的有效剂量分析（结合指数）进行分析。0.75-0.8 和 0.1-0.3 的联合指数分别显示中度协同和强烈协同(Chou and Talalay, Adv. Enz. Regul., 22, 27－55, 1984)。在 AraC 存在的情况下评价 VNP40101M 针对 L1210 白血病的细胞毒性效果，表 1 显示了 CLOETAZINE 与 AraC 对白血病的协同作用。

表 1：L1210 细胞暴露于 AraC 和 VNP40101M 的组合物 1 小时后的测量结果。

AraC (μM)	VNP40101M (μM)	受影响的部分	联合指数*
0.75	6	0.955	0.243
0.75	3	0.915	0.189
0.75	1.5	0.904	0.121
0.75	0.75	0.886	0.089
1.5	6	0.962	0.246
1.5	3	0.918	0.221
1.5	1.5	0.918	0.147
1.5	0.75	0.903	0.122
3	6	0.967	0.271
3	3	0.928	0.273
3	1.5	0.925	0.209
3	0.75	0.924	0.175
6	6	0.958	0.405
6	3	0.943	0.357
6	1.5	0.933	0.325
6	0.75	0.925	0.312

实施例 2、VNP40101M 和氟达拉宾对体外肿瘤细胞系的细胞毒性

通过细胞活性分析来测定 VNP40101M 和核苷类似物的组合物对肿瘤细胞系的细胞毒性。将多个白血病细胞系（L1210 和 HL-60）和淋巴瘤细胞系（Raji 和 Namalwa）暴露于单独的或与各种浓度氟达拉宾组合的 VNP40101M。72 小时后，通过测量线粒体氧化还原酶活性定量剩余的存活的细胞数量。所用的氟达拉宾浓度为 0.1-100μM，所用的 VNP40101M 浓度为 0.1-100μM。对显示 VNP40101M 和氟达拉宾联合效果的有效剂量分析（结合指数）进行分析。0.75-0.8 和 0.1-0.3 的联合指数分别显示中度协同

和强烈协同。

实施例 3、VNP40101M 和 AraC 对白血病的体内抗肿瘤活性

在 0 天将 0.2mL 磷酸盐缓冲盐水（PBS）中的 1×10^6 的 L1210 细胞腹膜内（ip）接种至 80 只 Balb/c×DBA/2（CD2F1）雌性小鼠。将小鼠随机地分成 6 组，每组 10 只小鼠。这些动物不被治疗或在第 1 天用 5 或 10mg/kg 的单次大丸剂量 VNP40101M 腹膜内给药治疗，在第 1、3、5、7 和 9 天一起使用或不使用 AraC（50mg/kg，ip）。在试验期间，每日观察小鼠数次。将接种 L1210 细胞后存活 60 天以上的小鼠确定为长期生存者。产生了 Kaplan-Meier 孔，并用学生 T-测试（student T-test）分析动物的生存时间。显著性定义为 $P<0.05$。

表 2 显示对小鼠进行的 1×10^6 的 L1210 白血病细胞的腹膜接种导致了腹水的快速发展，接着未治疗控制的所有动物在 18 天内死亡而用 AraC 治疗控制的动物在 30 天内死亡。以 5 和 10mg/kg 的 VNP40101M 单次剂量治疗分别增加长期生存者到 50%和 90%。组合治疗要好于单试剂的治疗效果。5 和 10mg/kg 的 VNP40101M 加上 AraC 的治疗分别增加了长期生存者到 80 和 100%。我们的结果表明 VNP40101M 显著地增强了 AraC 的抗肿瘤效果。

表 2、单试剂和组合试剂的治疗效果比较

组	长期生存者（%）	P 值*
1. 未治疗控制	0	
2. AraC 50mpk	0	
3. VNP40101M 5mpk	50	
4. VNP40101M 10mpk	90	
5. AraC 50mpk+VNP40101M 5mpk	80	0.0024
6. AraC 50mpk +VNP40101M 10 mpk	100	0.0022

*VNP40101M 以相应的剂量对应于组合治疗

实施例 4、VNP40101M 和氟达拉宾对白血病的体内抗肿瘤活性

在 0 天将 0.2mL 磷酸盐缓冲盐水（PBS）中的 3×10^6 的 L1210 细胞腹膜内（ip）接种至 80 只 Balb/c×DBA/2（CD2F1）雌性小鼠。将小鼠随机

地分成6组，每组10只小鼠。这些动物不被治疗或在第1天用5或10mg/kg的单次大丸剂量VNP40101M腹膜内给药治疗，在第1—9天中的几天一起使用或不使用氟达拉宾（70mg/kg，ip）。在试验期间，每日观察小鼠数次。将接种L1210细胞后存活60天以上的小鼠确定为长期生存者。产生了Kaplan-Meier孔，用学生T-测试分析动物的生存时间。显著性定义为P<0.05。

表3显示对小鼠进行的3×10^6的L1210白血病细胞的腹膜接种导致了腹水的快速发展，接着未治疗控制的所有动物在18天内死亡而用氟达拉宾治疗控制的动物在30天内死亡。用10mg/kg的VNP40101M单次剂量治疗增加了长期生存者到40%，组合治疗要好于单试剂的治疗效果。10mg/kg的VNP40101M加上氟达拉宾的治疗增加了长期生存者至90%。我们的结果表明VNP40101M显著地增强了氟达拉宾的抗肿瘤效果。

表3、单一试剂和组合试剂的治疗效果比较

组	长期生存者(%)	P值*
1. 未治疗控制	0	
2. Fluda 70mpk	0	
3. VNP40101M 5mpk	0	
4. VNP40101M 10mpk	40	
5. Fluda 70mpk+VNP40101M 5mpk	0	
6. Fluda 70mpk +VNP40101M 10 mpk	90	0.0025

实施例5、VNP40101M和AraC治疗AML患者的用途

以0.5-4.0gm/m^2/day的剂量在1-6天静脉连续输注给药AraC。优选地，在3-4天给药AraC的剂量为1.0-2.0gm/m^2/day。第1-3天AraC输注后给药VNP40101M 15-30分钟以上，VNP40101M的剂量为200-700mg/m^2。优选地，AraC输注后1天给药VNP40101M，而且剂量为450-650mg/m^2，重复多次循环治疗。

实施例6、VNP40101M和氟法拉滨治疗AML患者的用途

以10-50gm/m^2/day的剂量在1-6天静脉连续输注给药氟法拉滨。优选地，连续5天给药氟法拉滨的剂量为30-40gm/m^2/day。氟法拉滨给药

之前、期间、或之后单次大剂量注射给药 VNP40101M 15-60 分钟以上，VNP40101M 的剂量为 200-700mg/m^2，优选地，VNP40101M 的剂量为 450-650mg/m^2，而且，重复多次循环治疗。

实施例 7、VNP40101M 和替匹法尼治疗 AML 患者的用途

以 200-800gm/剂、每天 1-3 剂口服给药替匹法尼直到 28 天。优选替匹法尼以 600gm/剂给药，每天 2 剂，连续 28 天。替匹法尼给药之前、期间、或之后单次大剂量注射给药 VNP40101M 15-60 分钟以上，VNP40101M 剂量为 200-700mg/m^2，优选地，VNP40101M 的剂量为 450-650mg/m^2，重复多次循环治疗。

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